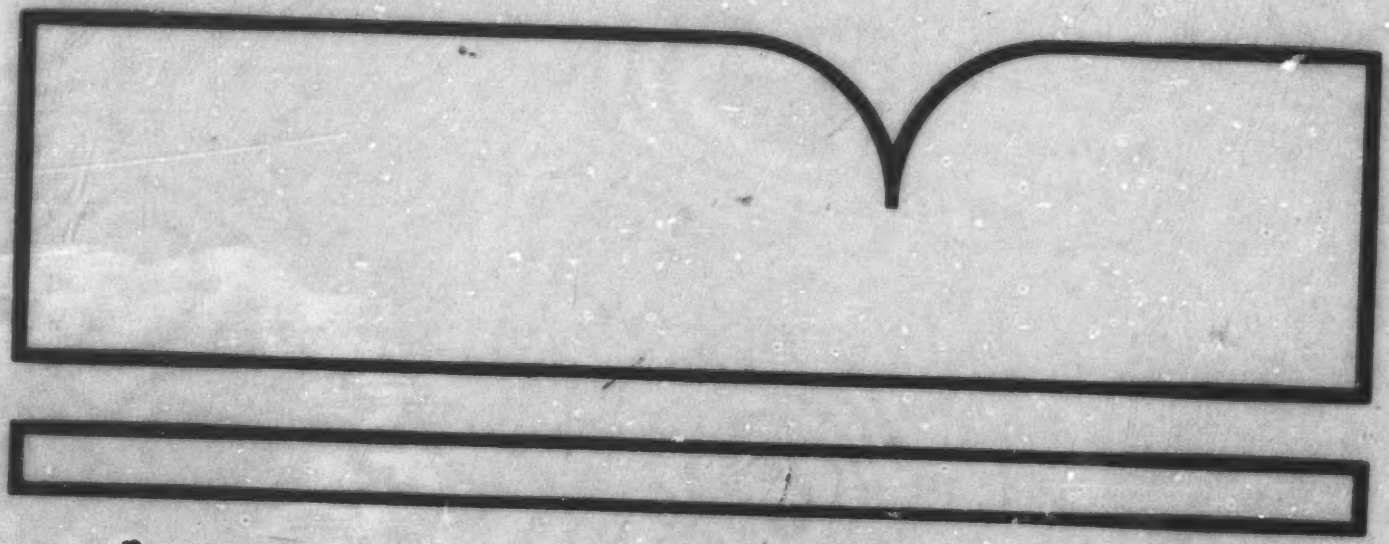
PB85-104941

Pulmonary Function Testing in Small Laboratory Mammals

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1984



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-84-112	2.	3. PB85-104941
4. TITLE AND SUBTITLE Pulmonary Function Testing in Small Laboratory Mammals (Journal Version)		5. REPORT DATE 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) John J. O'Neil and James A. Raub		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Inhalation Toxicology Division Health Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. AAGA1D 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES Environmental Health Perspectives Vol. 56,pp. 11-22, 1984		

16. ABSTRACT

The lung is the primary organ likely to be exposed by inhalation studies and, therefore, measurement of changes in lung function are of particular interest to the pulmonary physiologist and toxicologist. Tests of pulmonary function have been developed which can be used with small animals to measure spirometry (lung volumes), mechanics, distribution of ventilation, gas exchange or control of ventilation. These tests were designed on the basis of similar tests which are used in humans to diagnose and manage patients with lung disease. A major difference is that many of the measurements are performed in anesthetized animals, while human pulmonary function is usually measured in awake cooperating individuals. In addition, the measurement of respiratory events in small animals requires sensitive and rapidly responding equipment, because signals may be small and events can occur quickly. In general, the measurements described provide information on the change in normal lung function which results primarily from structural changes. These tests of pulmonary function can be repetitively and routinely accomplished and the results appear to be highly reproducible. Although some are quite sophisticated, many can be undertaken with relatively inexpensive equipment and provide useful information for toxicological testing.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 12
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-84-112
JOURNAL ARTICLE

Environmental Health Perspectives
Vol. 56, pp. 11-22, 1984

Pulmonary Function Testing in Small Laboratory Mammals

by John J. O'Neil* and James A. Raub*

The lung is the primary organ likely to be exposed by inhalation studies and, therefore, measurement of changes in lung function are of particular interest to the pulmonary physiologist and toxicologist. Tests of pulmonary function have been developed which can be used with small animals to measure spirometry (lung volumes), mechanics, distribution of ventilation, gas exchange or control of ventilation. These tests were designed on the basis of similar tests which are used in humans to diagnose and manage patients with lung disease. A major difference is that many of the measurements are performed in anesthetized animals, while human pulmonary function is usually measured in awake cooperating individuals. In addition, the measurement of respiratory events in small animals requires sensitive and rapidly responding equipment, because signals may be small and events can occur quickly. In general, the measurements described provide information on the change in normal lung function which results primarily from structural changes. These tests of pulmonary function can be repetitively and routinely accomplished and the results appear to be highly reproducible. Although some are quite sophisticated, many can be undertaken with relatively inexpensive equipment and provide useful information for toxicological testing.

Introduction

Tests of pulmonary function have been developed for use in small laboratory animals. Many aspects of lung function in small animals, including spirometry, mechanics, distribution of ventilation, gas exchange and ventilatory control, can be evaluated. These tests were designed on the basis of similar tests which are used in humans to diagnose and manage patients with lung disease. The major difference is that many of the measurements are performed in anesthetized animals while human pulmonary function is usually measured in awake cooperating individuals.

Structure and function are tightly coupled, and the presence, site and extent of pulmonary disease in small animals can be estimated. Changes in pulmonary function should reflect alterations in normal structure. Pulmonary function tests have been found to be sensitive to changes in the lungs of experimental animals and in some cases show evidence of damage before it is possible to detect it histologically. The measurement of respiratory events in small animals requires sensitive and rapidly responding equipment. Signals may be small, and events can occur quickly. The frequency response of the measuring equipment, pressure transducers, and electronic amplifiers needs to be carefully evaluated to assure that an accurate reproduction of the respiratory events can be recorded. In general, the measurements discussed in this paper provide information on the change in normal lung function which results primarily from structural changes. These tests of pulmonary function can be accomplished on a routine basis should one wish to use them for toxicological testing. Although some are quite sophisticated, many can be undertaken with relatively inexpensive equipment and provide useful information.

Pulmonary physiology is well described in several excellent texts (*1–5*), and we assume that most of the underlying principles upon which these tests are based can be obtained from them. We will briefly discuss some of the techniques that can be used to make respiratory measurements in small animals, but refer the reader to the available literature for more in-depth discussions of the principles and techniques upon which these measurements are based. The literature on pulmonary function testing in laboratory animals is extensive so we have chosen to cite studies on small animals such as rats, hamsters, and mice.

Measurement of Lung Volumes

It is convenient to describe the gas contained in the lungs in terms of four independent volumes and four capacities (*2*). Lung capacities are the sum of two or more lung volumes. These relationships are illustrated in Figure 1.

In man, total lung capacity (TLC) is the amount of

*Clinical Research Branch and Toxicology Branch, Inhalation Toxicology Division, Health Effects Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC 27711.

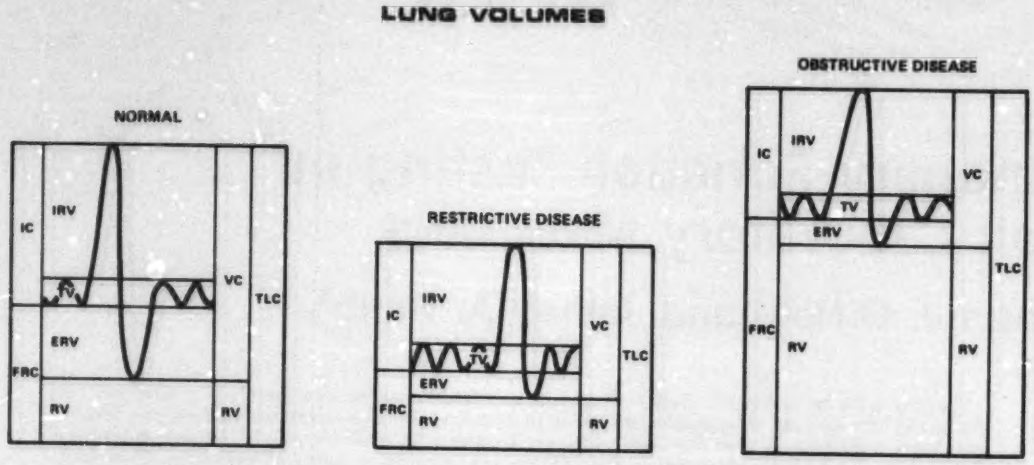


FIGURE 1. Schematicized tracing of breathing as recorded by a spirometer to illustrate the volumes and capacities commonly defined in mammalian lungs and the changes which might occur to lung volumes in the presence of restrictive or obstructive pulmonary disease. The lung capacities are the sum of two or more of the four primary volumes in the lungs. ERV = expiratory reserve volume; FRC = functional residual capacity; IC = inspiratory capacity; IRV = inspiratory reserve volume; RV = residual volume; TLC = total lung capacity; TV = tidal volume; VC = vital capacity.

gas in the lungs at the end of a maximal inspiration, while residual volume (RV) is the amount of gas in the lungs at the end of a maximal expiration. In experimental animals, these volumes are defined at specific static pressures, following inflation or deflation. TLC is measured at a particular total respiratory system pressure (P_{rs}), lungs and chest wall, defined as the pressure at the airway opening (P_{ao}) minus barometric pressure (P_B), or transpulmonary pressure (P_L), lungs only, defined as P_{ao} minus the plural pressure (P_{pl}). The pressures used to define TLC by various investigators fall in the range of 25 to 35 cm H_2O. The RV in small mammals is usually defined as the volume of gas remaining in the lungs after all available gas has been removed by lowering the P_{ao} to some point below P_B. The airway pressure required to remove all the available gas needs to be determined experimentally. Because they have compliant chest walls (*6*), small animals usually require that P_{ao} be lowered only 15 to 20 cm H_2O below P_B. The pressures at which measurements of TLC and RV are made should always be reported.

In small animals, vital capacity (VC) can be determined by measuring the volume necessary to inflate the lungs from RV to TLC. This is conveniently done by measuring the volume displaced from a syringe as the lungs are inflated or by placing the animal in a plethysmograph and determining the VC from volume changes.

Thoracic gas volume at RV and at the functional residual capacity (FRC) has been successfully measured in small mammals by using at least three different techniques. Perhaps the easiest technique, which is also used in man, is that which measures the dilution of an inert tracer gas contained in a reservoir which is equilibrated with the gas in the lungs (*4*). A nitrogen dilution technique based on this principle has been described by King (*7*) for measuring FRC in rats; however, this is a fairly tedious and time-consuming method. More recently, neon (Ne) dilution techniques have been used by Takezawa and his co-workers (*8*) to measure RV and FRC with a test gas containing 0.5% Ne. A volume equivalent to the previously determined VC was exchanged with the gas in the lungs 10 times from RV. The final Ne concentration in the gas sample was analyzed and RV was calculated on the basis of the Ne dilution. FRC was determined in a similar manner by using a volume equivalent to the inspiratory capacity and injecting the gas at end expiration.

A second method for measuring FRC uses Boyle's law. It is based on the concept that the volume of a fixed quantity of gas at a constant temperature varies inversely with the absolute pressure. If the airway of an animal is occluded at end expiration and the animal makes breathing attempts, the pressure and volume in the thorax and abdomen are changed. The net changes in gas volume can be measured with a body plethysmograph, and the corresponding changes in P_{ao} can be measured with a pressure transducer. The original volume of thoracic gas at the time of airway obstruction (i.e., FRC) can then be calculated from a knowledge of P_B and by correcting for the volume-pressure behavior of the equipment connected with the airway.

The Boyle's law method was originally adapted for use with rats by Palacek (*9*). Subsequently, Koo and his co-workers (*10*) used a pressure plethysmograph to measure FRC in hamsters; this system was used by Snider et al. to study animals with experimental emphysema (*11*) and experimental fibrosis (*12*). Recently, Sinnett and his colleagues (*13*) have described a plethysmograph which can be used to measure the

volume changes associated with the Boyle's law maneuver in animals as small as mice.

The measurement of FRC by body plethysmography is suited to screening in toxicological studies because it is easy to perform and can be repeated as frequently as desired. It is generally accepted that the Boyle's law technique measures the total gas contained in the thorax. However, there is potential for errors if abdominal gas volume is large. In at least one study with rats, Lai (*14*) has shown that the contribution of abdominal gas to the Boyle's law measurement of FRC is small. In disease states when some thoracic gas may be trapped in bullae or behind closed airways, gas dilution techniques could underestimate the volume of gas in the lungs. Theoretically, if both gas dilution and the Boyle's law techniques are used together, it might be possible to determine the amount of gas trapped behind closed airways or in bullae, although we know of no studies to date that have demonstrated this in small animals.

A third method, oxygen absorption atelectasis, has been used to estimate FRC (*15*). If an animal is ventilated with 100% O_2 until minimal amounts of N_2 remain in the lungs and the airway is then occluded at end expiration, all of the gas in the lung will be absorbed into the blood, and the lungs will collapse. This change in volume can be measured plethysmographically. Because small animals have such compliant chest walls, they appear to tolerate complete atelectasis without developing the unduly large negative intrathoracic pressures and associated circulatory complications that would kill an animal with a stiffer chest wall. This technique can also be applied to measure TLC if, after the absorption of oxygen from the lungs, the volume change is measured during inflation to TLC (*6*). Oxygen absorption atelectasis is also a useful method for degassing lungs *in situ* (*16*).

Some static measurements of lung volume (RV, VC, and TLC) are useful indicators of structural changes in the lungs and have been determined routinely in man and animals. On the other hand, FRC in small animals is largely set by dynamic mechanisms such as breathing frequency, inspiratory muscle tone or glottal braking (*17,18*) and consequently is influenced by anesthesia, reflex activity, intubation and equipment resistance. For example, FRC may fall remarkably during the transition from the awake to the anesthetized state (*17*). When emphysema is present, TLC and FRC are increased, and the extent to which these volumes change is related to the severity of the disease (*19*). Interstitial lung diseases such as fibrosis can cause significant decreases in FRC, RV and TLC (*12*). The types of changes in lung volumes that might occur in pulmonary disease are illustrated in Figure 1.

Tests of the Distribution of Ventilation

Total ventilation may be normal, but inspired gas may not be distributed uniformly in the lung. Regional differences in lung ventilation result from a combination of static and dynamic factors, which include: the distribution of P_{pl} around the lungs (influenced by gravity and the shape of the lungs and the chest wall), the distribution of resistances and compliances in the lungs, airway closure, and the occurrence of expiratory flow limitation in the airways. Many diseases produce changes which influence these factors in the lungs and can lead to maldistribution of ventilation. There are several tests which are affected by changes in the distribution of ventilation and which might be useful in detecting pulmonary disease in small mammals, though the experience with them to date is limited.

Single Breath Oxygen Test (Closing Volume)

Information about the volume at which airways close can be obtained if, following a single inflation to TLC with 100% oxygen, expired volume is continuously plotted versus the expired nitrogen concentration measured at the airway opening (Fig. 2). The first gas washed out of the lungs is dead space (V_D) gas which contains little or no N_2 (phase I). Nitrogen concentration then rises sharply as V_D is washed out and alveolar gas begins to appear at the airway opening (phase II). This phase is followed by a slow increase in the concentration of N_2 (the alveolar plateau) which continues over most of the expired volume (phase III) and which is composed of the expired gas from many alveolar units. Finally, as units with low N_2 concentration decrease or stop their contribution to the expired gas, there is a marked increase in the N_2 concentration (phase IV). The onset of phase IV is thought to represent the progressive closure of small airways. This volume difference from RV is called the closing volume.

This test is believed to indicate airway closure in humans and is relatively easy to perform; however, it has not been widely adapted for use with small mammals. Likens and Mauderly (*20*) have performed this test in rats in a plethysmograph by measuring lung volume change and the nitrogen concentration at the

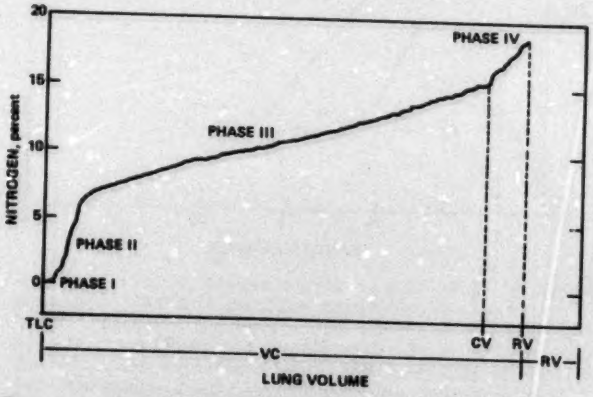


FIGURE 2. Single breath oxygen test. Airway nitrogen concentration of a rat measured versus volume change following a single breath of 100% oxygen. See description in text. Redrawn with permission from Sue Loscutoff, Richland, WA (unpublished observations).

airway opening with a nitrogen meter. They studied normal rats and rats which had experimentally produced emphysema. In the presence of pulmonary emphysema, the slope of phase III did not change, while the closing volume was significantly increased compared to the controls. Although consistent with observations made in humans, the mechanisms of airway closure is poorly understood.

Multiple Breath Nitrogen Washout

This test is performed while an animal is ventilated with 100% O_2 (*19,21*). With each O_2 breath, the concentration of expired N_2 decreases until only N_2 being washed from tissue stores is exhaled (Fig. 3). The log of peak expired N_2 concentration when plotted versus breath number is usually characterized by a straight line. The number of breaths required to reach an N_2 concentration of less than 2% (the breath index) has been used to characterize N_2 washout. Hamsters which have experimental emphysema show marked changes in the slope of the nitrogen washout (Fig. 3) and in the breath index (*19*). The nitrogen washout is slowed by the increased ratio of FRC to tidal volume (V_T) as well as nonuniform time constants among individual lung units. Nitrogen washout has been normalized for lung volumes in man (*22,23*), and it may be possible to apply similar procedures to measurements with small animals.

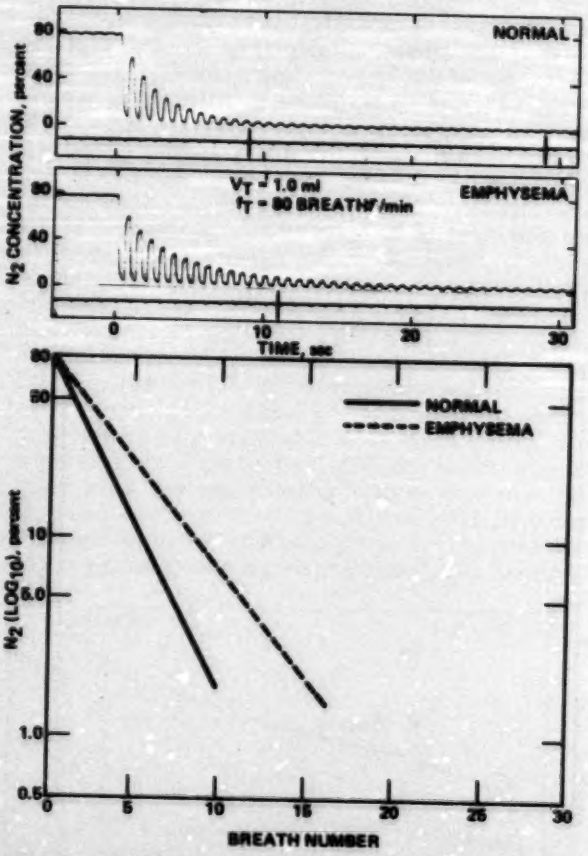


FIGURE 3. Multiple breath nitrogen washout. Airway nitrogen concentration measured during ventilation with 100% oxygen in a normal hamster and in a hamster which has experimental emphysema (*19*). Nitrogen concentration is normally about 80% and during inspiration of 100% oxygen it falls to near zero. With each breath the nitrogen remaining in the lungs is diluted until only body stores of nitrogen are being removed. The log of peak nitrogen concentration is plotted versus breath number in the lower panel to describe the rate at which nitrogen is removed from the lungs.

Tests of Pulmonary Gas Exchange

Diffusing Capacity for Carbon Monoxide

It is possible to test the function of the alveolar-capillary membrane by measuring the rate at which a test gas, such as carbon monoxide (CO), diffuses into the blood from the airspaces. Because CO is avidly bound by hemoglobin, its partial pressure in the pulmonary capillary blood remains essentially zero. If the concentration of CO can be determined in the air spaces, it is possible to establish the partial pressure difference that exists across the alveolar-capillary barrier. The diffusing capacity measurement is based on the rate at which CO disappears from the lungs. Takezawa and his co-workers (*8*) described one method which can be used in anesthetized small animals to measure the single breath diffusing capacity of the lungs for carbon monoxide ($D_{L_{CO}}$). The technique was used in conjunction with the gas dilution method described for measuring RV and FRC. The lungs were inflated from RV to TLC (the vital capacity) with a volume of gas which contained 0.5% CO and 0.5% Ne. The gas was maintained in the lungs for a 10-sec period of breath holding, after which approximately 50% of the gas was withdrawn to wash out the anatomical and equipment dead space. The remainder of the gas was collected immediately into a second syringe as an "alveolar" sample. This latter sample was analyzed for Ne and CO concentrations on a gas chromatograph and the diffusing capacity calculated using standard formulas. Other methods to measure the diffusing capacity (e.g., by rebreathing techniques) have been adapted for use with rats by Turick (*24*) and by Johanson and Pierce (*25*). The rebreathing methods were modified for use with hamsters by Snider and his co-workers (*12*). The single breath method is somewhat easier to use and is highly reproducible; however, both techniques appear to be sensitive to subtle changes in pulmonary structure.

There are several factors which can affect the diffusing capacity. For example, the $D_{L_{CO}}$ will be reduced if well-ventilated airspaces are poorly perfused, or vice-versa, that is, the distribution of ventilation to perfusion ratios ($\dot{V}_A/\dot{Q}$ ratio) is abnormal; if gas exchange tissue is lost, as occurs with the destruction of alveolar

walls in pulmonary emphysema; if the thickness of the alveolar to capillary barrier is increased to the point that diffusion is impaired; or if pulmonary capillary blood volume changes.

The $D_{L_{CO}}$ decreases in a dose-dependent manner when varying degrees of experimental pulmonary emphysema are present (*19*). It is decreased in rats which have pulmonary asbestosis (*26*). There is, in addition, a high correlation between quantitative morphometric determinations of structural changes in the lungs and the functional measurement of $D_{L_{CO}}$ (*26,27*). Similarly, there is a high correlation between the body size of the animal and the measurement of the $D_{L_{CO}}$ (Fig. 4).

Blood Gases

If gas exchange is impaired, CO_2 may accumulate and oxygen tensions may be low. The body can compensate for some abnormalities such that the partial pressure of carbon dioxide P_{CO_2} or pH of blood might remain within normal limits, even when severe impairment of gas exchange exists. For example, increased V_D/V_T can be compensated by increased minute ventilation to maintain normal alveolar ventilation and P_{CO_2}; the kidney can also defend pH by maintaining a compensatory metabolic alkalosis in the face of chronic hypercarbia. The measurement of blood gases is useful for evaluating both acute and chronic conditions related to pulmonary dysfunction or disease and for studies on the control of ventilation. Using implanted catheters, it is possible to measure acid base imbalances, increases or decreases in the P_{CO_2} or the partial pressure of oxygen (P_{O_2}), as well as the differences in the P_{O_2} existing between the alveolar gas and the arterial blood. This last measurement can be used to estimate pulmonary shunt blood flow (blood flow past nonventilated alveoli). O'Brien and his colleagues (*28*) developed techniques to measure blood gases in hamsters both at rest and during exercise on a horizontal treadmill. Lucey et al. (*29*) used these techniques to compare the blood gases in normal and emphysematous hamsters. And Lai and his co-workers (*30*) have measured the changes in blood gases that result from the acute inhalation of CO_2 in the rat.

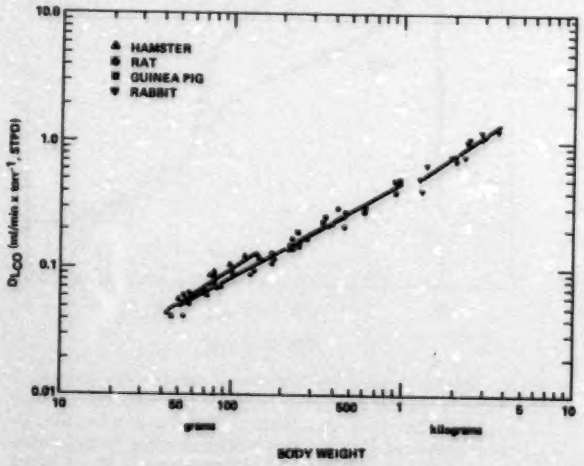


FIGURE 4. Plot of the single breath diffusing capacity for carbon monoxide in four species of small laboratory animals as a function of body mass (log-log plot). Reproduced with permission from Takezawa et al. (*16*).

Pulmonary Mechanics

The mechanical behavior of the respiratory system is determined by its static and dynamic properties. In general, static properties are measured by static or quasi-static volume–pressure curves during inflation or deflation (*31*). Dynamic properties are determined with measurements of flow–volume curves, airways resistance and compliance and total respiratory system impedance. The mechanical behavior of the respiratory system is influenced by the elastic structural nature of the tissue, surface forces acting at the air–liquid interface of the alveolar surface, airway smooth muscle activity or mucus secretion which may affect airway caliber and influence airway resistance, and skeletal muscle activity in the chest wall which may change total respiratory system compliance.

Static Properties

By analogy with length-tension curves for linear elastic materials, volume–pressure curves express the static properties of volume elastic structures. Volume–pressure curves of the lungs and chest wall, of lungs alone or of the chest wall alone can be made by using either plethysmographic or spirometric measures of volume change, in combination with pressures measured at the airway opening, body surface (P_{bs}), and the pleural or esophageal space.

Quasi-static volume–pressure curves of the total respiratory system is anesthetized intact small animals can be constructed by measuring the pressure differences across the lungs and chest wall as the lungs are slowly inflated to TLC and then deflated to RV. Volume change can be measured with plethysmographic or spirometric techniques, and pressure change can be measured with appropriate differential pressure transducers. An example of the quasi-static volume–pressure relationship of the lungs and chest wall from a normal hamster and a hamster with experimental emphysema is shown in Figure 5. Because small animals have compliant chest walls, this curve resembles the volume–pressure behavior of the lungs alone. In order to partition pulmonary and chest wall compliance, pleural pressure is estimated using a saline-filled catheter which is placed in the thoracic esophagus. Care is required in the placement of an esophageal catheter to avoid potential errors (*14,32*). Koo and his co-workers (*10*), Palacek (*9*), Diamond and O'Donnel (*33*), Lai and Hildebrandt (*34*), and Sinnett et al. (*13*) have all described techniques which can be used

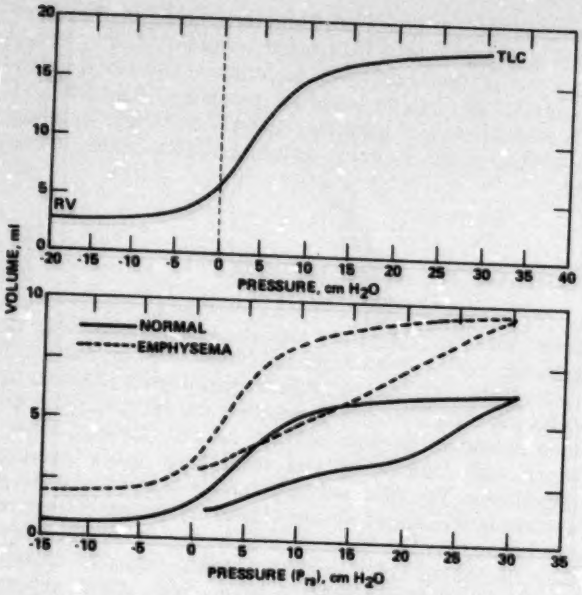


FIGURE 5. Volume–pressure relationships of the lungs and chest wall. Airway pressure, measured with a pressure transducer, is plotted versus lung volume, measured in a pressure plethysmograph: (top) deflation limb from the volume–pressure curve of a normal rat; (bottom) changes in the volume–pressure relationships of hamsters which develop experimental pulmonary emphysema (*19*).

to measure quasi-static volume–pressure curves in intact small animals.

Volume–pressure curves can be easily constructed on excised lungs and several investigators have made useful observations using simple equipment (*35–37*). Such curves are usually constructed from the degassed state. Degassing can be accomplished (once) before the lungs are excised (*15,16*) by oxygen absorption atelectasis (this requires a functioning circulation) or by exposing the excised lungs to very low pressures in a vacuum desiccator (*16*). Volume change can be measured in a plethysmograph or by volume displacement from a syringe. Pressure change is commonly measured by using a water manometer with corrections applied for gas compression and volume displacement. Use of a differential pressure transducer usually eliminates the need for correction factors. Frazer and Weber (*38,39*) have described an elegant method for measuring volume–pressure curves of excised rat lungs and have used these techniques to examine the mechanics of gas trapping in the lungs. Freeman et al. (*35*) have reported changes in the volume–pressure relationships of lungs excised from rats exposed to NO_2 which developed mild emphysema. It is also possible to demonstrate the differences between tissue recoil forces and the remarkable events which occur at the alveolar air–liquid interface during respiration by constructing volume–pressure curves with air filled as compared to saline filled lungs (*40*). Hayatdavoudi and his co-workers (*36*) reported air and saline volume–pressure curves on lungs excised from rats which had been exposed to 60% O_2 for 7 days. They demonstrated changes in the elastic properties of the lung tissue as opposed to changes in surface forces. In addition, they confirmed changes in lung volumes which had been measured by using the gas dilution techniques described by Takezawa et al. (*8*) and which were not apparent histologically in their study.

Dynamic Properties

During forced expiration, maximum airflow is set by flow-limiting mechanisms which are influenced by airways resistance and gas density. When maximum flow is plotted against volume, a maximum expiratory flow–volume (MEFV) curve can be generated (Fig. 6). When airways disease is present or lung recoil is lost, flow at any volume may be reduced compared to normal flows, and the shape of the MEFV curve may change (*41*). In anesthetized small mammals, the MEFV curve requires that the animals be intubated. The maximal expiratory effort is performed by abruptly exposing the airway of

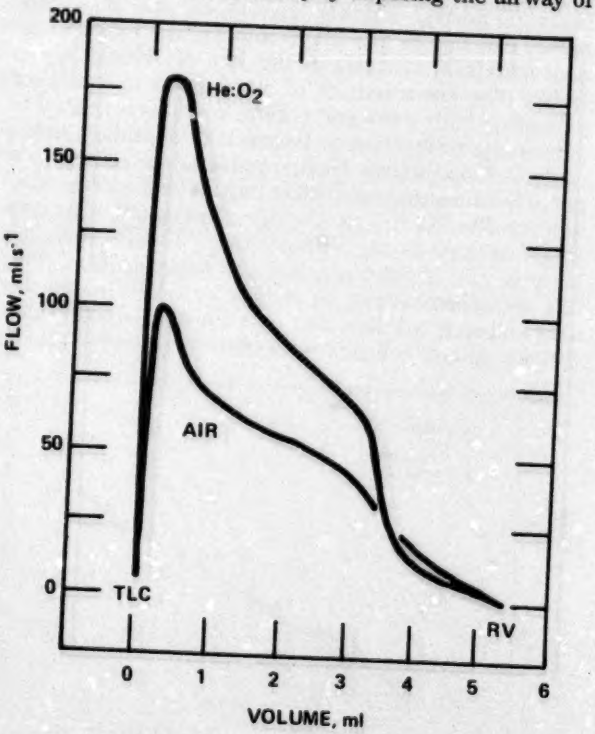


FIGURE 6. Maximal expiratory flow volume curves measured from a 120 g hamster during ventilation with air and with 80% helium: 20% oxygen. The curve has been redrawn and smoothed for illustrative purposes. These curves were generated by measuring flow in a rapidly responding "flow" plethysmograph. The point at which the two curves superimpose is referred to as the isovolume point. In actual practice, several curves would be recorded and ensemble averaged to develop a smooth curve (J. J. O'Neil, Chapel Hill, NC, and D. E. Leith, Boston, MA, unpublished observations).

the animal to a partial vacuum. The technique to perform and measure such fast events requires specialized equipment with very rapid response times.

Diamond and O'Donnell (*33*) measured MEFV curves in rats using an integrated flow plethysmograph. Lucey and his co-workers (*32*) measured MEFV curves in hamsters using a pressure plethysmograph. Recently, Damon et al. (*42*) using a flow plethysmograph have measured MEFV curves in normal rats and in rats with experimental emphysema. They reported that flows at all volumes were lower in rats with experimental emphysema when compared to control animals and that peak flows at the onset of the maneuver were also reduced.

Additional information can be obtained regarding small airways function by performing the MEFV maneuvers during ventilation with gases other than air. A mixture of 80% helium and 20% oxygen (He:O_2), which is less dense and slightly more viscous than air, is commonly used for this purpose (*43,44*). Flows over most of the curve will be greater with He:O_2 than with air. However, in small airways, where flow is normally laminar, the pressure drop is independent of gas density; therefore, flow in these small airways is less affected. The MEFV curves generated in air and He:O_2 generally intersect in the region where flow becomes laminar. The volume at which this occurs is called the volume of isoflow (Fig. 6).

Oscillatory Mechanics

Total respiratory system impedance has been measured in rats by Jackson and Watson (*45*). They imposed sine wave oscillations on the normal breathing of anesthetized rats using a computer-controlled speaker device which was programmed to keep flows the same at all frequencies. They measured the frequency dependence of resistance and compliance as well as the resonant frequency (i.e., the frequency where reactance passes zero going from negative to positive). They used optimization techniques and concluded that the respiratory system of the rat behaves generally like a series mechanical network consisting of resistive, compliant, and inertive elements. Although many questions still exist regarding the interpretation of such observations, these tests offer hope of rapid and convenient measurements in assessing airways disease in experimental animals.

Tests of Ventilatory Control

Ventilation can be influenced by voluntary or automatic regulation. Voluntary regulation arises from cortical control of the automatic system and can modify respiration, for example, in anticipation of stress or exercise. Automatic regulatory control includes three main components: central neural control which processes and integrates information from various peripheral sources and higher neural regions; chemical control which detects levels of O_2, CO_2, and pH in the lung, the blood and the cerebrospinal fluid; and mechanical control which is mediated through reflexes predominately from the lung and the respiratory muscles. All of these influences can alter the output of impulses from the brainstem to the ventilatory apparatus. Therefore, one means of looking at "integrated" neural control is to measure the changes in total ventilation. Minute ventilation ($\dot{V}_E$) is the volume of gas that the lungs expire per minute and is the product of the rate (f) and the depth (V_T) of breathing. An analysis of ventilation can also include measurement of the total time of the average breath (T_{tot}), time for inspiration (T_i), and time for expiration (T_e). An index of the central inspiratory drive (CID) can be made by calculating V_T/T_i, and an index of respiratory timing is T_i/T_{tot}. Both of these measurements are affected by changes in lung compliance, pulmonary resistance, and neuromuscular control. Ventilatory timing can be measured on an anesthetized animal in a pressure or flow plethysmograph and on unanesthetized animals in the barometric plethysmograph. Vizek and Palacek (*46*) have reported the effects of halothane on V_T and on the timing of the respiratory cycle in rats.

Another measure of CID, which is presumably independent of compliance and resistance and is reflective of respiratory muscle activity is the tracheal occlusion pressure. The airway of an experimental animal is mechanically occluded at the end of an expiration and airway pressure is measured 0.1 sec after the onset of an inspiration. In man, loss of respiratory drive is associated with a decrease in the occlusion pressure. Lai and his colleagues (*30*) have described techniques which can be used to measure the occlusion pressure in rats; however, we are not aware of any such measurements made in small animals with lung disease or following treatment with toxic compounds.

Ventilatory Response to CO_2

A CO_2 response curve is obtained by measuring ventilation of an animal while it breathes increasing concentrations of CO_2 in oxygen. The concentration of CO_2 is increased in a stepwise fashion, and ventilation is measured at each point. Responsiveness is determined from the slope of the change in $\dot{V}_E$ for the given change in end tidal P_{CO_2}. Measurements of the ventilatory response to CO_2 of unanesthetized hamsters (*47*) and guinea pigs (*48*) have been reported over a wide range of CO_2 concentrations. Lucey (*29*) and Lai (*49*) and their co-workers have recently described techniques for use with unanesthetized animals to concomitantly measure blood gases and the ventilatory response to CO_2 inhalation. Also, Chvalova et al. (*50*) observed that the arterial P_{CO_2} was lower and the response to CO_2 inhalation was depressed compared to controls in rats that had experimental silicosis.

Brain stem respiratory neurons are also influenced by mechanoreceptors. The majority of these mechanorecep-

tors are located in the lungs and airways but there are others in the respiratory muscles (chestwall, abdomen, and diaphragm), blood vessels, and possibly the joints and skeletal muscles of the limbs. A method of testing reflex control which takes advantage of the Hering-Breuer reflexes in the lung has been described by Gillespie et al. (*51*). Inflation of the lung causes an inhibition of central inspiratory drive while lung deflation causes increased ventilation. By rapidly decreasing or increasing the pressure surrounding an animal in a body plethysmograph, lung volume was changed resulting in initiation of these reflexes. Gillespie (J. R. Gillespie, Davis, CA, personal communication) has subsequently found that exposure to ozone increased the time of apnea following a rapid inflation and also changed the timing of ventilation in rats.

Equipment

The measurement of respiratory events in small animals requires equipment capable of amplifying small and rapid signals. As with all dynamic measurements, the equipment must meet the criteria for amplitude and phase linearity and frequency response. Amplitude linearity is obtained when input and output are proportional. Phase linearity means that there is no time distortion in the signal. Frequency response is generally described in terms of bandwidth (i.e., the maximum and minimum frequencies at which the instrument can measure with defined levels of accuracy). There are several helpful references that discuss these criteria as they apply to biological measurements (*52–58*).

A Fleisch pneumotachograph attached to the airway of a small animal may have a larger dead space volume than the tidal volume of the animal. Measurement of breathing frequency or tidal volume with such a pneumotachograph could therefore result in hypercarbia and hypoxia unless efforts are taken to eliminate these effects (e.g., a bias flow of air or oxygen). In an effort to reduce equipment dead space and to minimize gas compliance in a measurement system, it is tempting to use tubing and valves which have a small internal diameter and hence a small volume. However, the resistance in small bore tubing may be high relative to that in the airway of the animal. High equipment resistance and use of bias flow can change airway pressure, end expiratory lung volume, and ventilation. In addition, when small bore tubing is used to connect to pressure transducers, inertial influences can distort the pressure measurement. Jackson and Vinegar (*56*) have reported on these and other effects with a number of transducers commonly in use today. In general, the rule of thumb for all tubing, fittings, valves, and connectors where rapid responses are important is "short and wide-bore."

Plethysmographs have been developed that are sensitive and which have frequency response characteristics which make them suitable for use with small animals. They are basically of two types: pressure plethysmographs and volume plethysmographs (*57*). In general, both are suitable for use with small animals but each has advantages for certain applications.

Pressure Plethysmographs

A pressure plethysmograph is a closed system such that, as the animal breathes to the outside, its thorax will alternately expand and relax, compressing and decompressing the gas inside the plethysmograph. The resulting pressure changes are measured with a sensitive pressure transducer. If gas compression in the system is either completely adiabatic or completely isothermal, the change in volume (dV) will be directly proportional to the change in pressure (dP) according to the following relationships:

$$dV = -(V/P)dP \quad \text{(for isothermal compressions)}$$

$$dV = -1.4(V/P)dP \quad \text{(for adiabatic compressions)}$$

Pressure plethysmographs are convenient to use because pressure and volume are directly proportional so long as the system is functionally isothermal or functionally adiabatic. Although volume changes during maximal expiratory maneuvers should be small relative to the total volume of the system, the gas temperature will tend to rise and fall (adiabatic heating and cooling) as the gas in the plethysmograph is compressed and decompressed. For example, the pressure signal produced by injection of 1 mL into an empty 1-L Plexiglas plethysmograph which is attached to a reservoir chamber of about 10 L will require approximately 30 to 40 sec to reach equilibrium. The initial pressure signal will be 40% higher than the final, if the plethysmograph contains only air (*13*). This occurs because the temperature rises when the gas is compressed and it takes 30 to 40 sec for this heat to dissipate into the walls of the plethysmograph. Since this loss of heat (pressure) is too fast to allow a plethysmograph of this size to be considered functionally adiabatic, the alternative is to try to make the system behave isothermally. The most commonly used method is to fill the reservoir attached to the animal chamber portion of the plethysmograph with a material which has a high heat capacity and a large surface area. Copper sponges have been used for this purpose. Such a material will quickly absorb and release the heat and the system will be nearly isothermal. The system described by Koo and his associates (*10*) is a good example of an isothermal pressure plethysmograph which has appropriate response characteristics for many measurements with small animals. For the purpose of eliminating thermal and atmospheric perturbations of the measuring system, the reference side of the differential pressure transducer should be connected to a similar sized chamber which is also filled with copper sponges. These investigators verified the response of their measuring system by "thumping" home a water-lubricated 2 mL syringe to generate a step change in

plethysmograph pressure. They adjusted a screw clamp placed between the plethysmograph and the transducer to damp oscillations from this signal and minimize overshoot. Another approach might be to use low-pass filters to discard any unwanted high-frequency signals.

Volume Plethysmographs

A volume plethysmograph is similar to the pressure plethysmograph, except that the plethysmograph has an opening to the outside which can be connected directly to a spirometer to measure volume change. A variation of the volume plethysmograph is to place a resistive element in the opening. Flow should be proportional to the pressure drop across this element. Several layers of 400 mesh stainless steel screen (oil-free and wrinkle-free) have been used as resistance elements because pressure drops are linear over a wide range of flows. Volume is obtained by electronically integrating this flow signal. The upper limits of frequency response achieved by "flow boxes" exceed that reported so far for pressure boxes. The system described by Sinnett and his co-workers (*13*) has excellent frequency response and is sensitive enough to measure the very small signals produced during a "Boyle's law measurement" of FRC in a mouse and fast enough to capture the information in a maximal expiratory flow volume maneuver.

Maximal expiratory flow volume curves are especially difficult to measure in small animals because the event occurs so rapidly. The time constant for MEFV curve slopes in 25-g mice is on the order of 19 msec (D. E. Leith and L. L. White, Boston, MA, personal communication) and specific portions of the MEFV curve will have even higher frequency content (e.g., peak flow at the onset of the curve). Measurement equipment should not overestimate high frequency information (overshoot) nor underestimate the curve (damping). Jackson and Vinegar (*56*) described techniques to test the response of differential pressure transducers and the associated amplifiers and recorders throughout the range of frequencies likely to be encountered when measuring respiratory function. Sinnett and his colleagues (*13*) have carefully discussed this problem with regards to plethysmographs for small animals. They have constructed and tested a plethysmograph–transducer–recording system which seems to meet all of the frequency response requirements to 240 Hz for such measurements. Their system was constructed for use with mice; however, they provide the principles to guide one in the construction of fast flow plethysmographs for use with larger laboratory animals such as hamsters, rats, and guinea pigs.

Barometric Plethysmograph

The barometric plethysmograph (Drorbaugh-Fenn box) permits the measurement of respiratory frequency and tidal volume in unanesthetized and unrestrained animals and would have distinct advantages for many toxicological studies, especially those relating to the control of breathing. If an animal is in a closed chamber the gas entering its lungs is warmed and humidified and therefore expands. This results in a pressure change in the system which is proportional to the tidal ventilation. The principles for such measurements were described by Drorbaugh and Fenn in 1955 (*59*). Several other authors (*47, 48, 60–64*) have discussed and used this technique which permits the measurement of the timing of ventilation, breathing pattern, and the CO_2 response, all without the stress of restraint or the depression associated with anesthesia. For example, Lucey et al. (*29*) made some interesting correlations between blood gases and breathing responses of hamsters to increased CO_2 in the barometric plethysmograph. Although it is probably adequate to measure relative changes in the frequency and tidal volume of quiet animals, the system is difficult to calibrate, gross breathing movements appear to disrupt the signal, and questions remain regarding the use of this system for making absolute measurements of ventilation.

Anesthesia

The barbiturates have been the most commonly used anesthetics in small laboratory mammals. Substances like sodium pentobarbital (Nembutal) are normally administered intraperitoneally with variable results depending on animal strain, age, size, and body fat content. However, barbiturates can depress respiratory and cardiovascular function.

Combinations of other injectable anesthetics have been tried. Ketamine hydrochloride (Vetalar) is effective and has been used in combination with a tranquilizer like acepromazine. Xylazine hydrochloride (Rompun) has been given in combination with this ketamine–acepromazine mixture; however, hypotension has been associated with its use in animals (*65*). Xylazine has also been used in combination with methohexital sodium (Brevital). Last, urethane, either by itself or in combination with chloralose and droperidol-fentanyl (Innovar-Vet) has been used with varying degrees of success in laboratory animals.

The most commonly used inhalation anesthetics are ether, nitrous oxide, halothane (Fluothane), and methoxyflurane (Metofane). Although they provide better control of the depth of anesthesia, they have disadvantages in that they may be toxic (*66*), and precautions must be taken when using such compounds on a regular basis in the laboratory environment.

Comments

All of the pulmonary function tests mentioned in this chapter are identical or similar to tests which have previously been used in man or other larger animals. The assumption has been made that the same physiological principles apply in small animals as apply in man.

Therefore, the interpretation of changes which occur in a pulmonary function test used in a small animal is basically that which would be applied to a human. However, there may be differences in the mechanisms which are the basis for these tests in small animals when compared to humans. For example, in upright humans it is thought that measurement of the closing volume by the single breath oxygen test is based on a pleural pressure gradient which exists between the apex and the base of the lung. This pressure difference is thought to be the result of gravity over the height of the lung. In small animals which may have a distance of less than two centimeters from the top to the bottom of their lungs, such a pressure gradient will be negligible. Nevertheless, Agostoni (*67*) reported measurement of a pleural pressure gradients in different animal species varying in size from rats to rams.

Small animals have very compliant chest walls; consequently, their lungs and chest walls can be additionally distended by increasing P_{ao} above those pressures normally used to measure TLC. Webb and Tierney (*68*) and Kao and Tierney (*69*) have reported that ventilation to high distending pressures can cause severe injury in the lungs of normal rats. Following exposure to toxic compounds the lungs of small animals may have increased sensitivity to these distending pressures. For example, Hayatdavoudi and his coworkers (*36*) observed that the lungs of rats which had been exposed to 60% O_2 for 7 days were more susceptible to the development of pulmonary edema following distention with high airway pressures.

Changes in pulmonary function have been measured even in the presence of mild lung disease. Several animal models of human pulmonary disease have been reported in the literature (*11,12,19,29,36,50*). For instance, pulmonary emphysema has been produced in hamsters by the intratracheal instillation of elastase (*13,19*). Raub and his colleagues have reported that changes in pulmonary function are related to the amount of elastase administered. Changes were measured even at the lowest doses of elastase which were used. Their data emphasize the sensitivity which exists with these pulmonary function tests.

Additional information on pulmonary function may be derived with the application of new tests. Seeherman and his associates (*70*) have described elegant methods which can be used to measure maximum oxygen consumption in exercising animals as small as the 7-g pygmie mouse. Such tests have not been applied in animal toxicology; however, the potential for their use should be explored.

Much of our understanding of normal human pulmonary physiology is derived from work done with animals. As one studies different sized animals (i.e., small to large) a proportionality exists in both structure and function (*6,27,71–74*). Extrapolation models need to be developed that relate physiological changes which occur in small animals to changes which might occur in large animals.

Conclusions

We have described pulmonary function tests which are in use today and which can be accomplished in the smallest common laboratory animals such as rats, hamsters, and mice. Because of the constraints imposed by equipment and measurement techniques these tests are the most difficult to use with small animals. Any test which can be accomplished in animals this small can be accomplished in larger laboratory animals (e.g., ferret, rabbit, cat, dog, and so on).

Because the lung is the primary organ likely to be exposed by inhalation studies, the tests of pulmonary function which have been described in this chapter are of particular interest to the pulmonary physiologist and toxicologist. The need for continued use of animal studies is clear: animal studies are relatively inexpensive, large and significant numbers of animals can be exposed under controlled circumstances, and studies can be undertaken which would otherwise be impossible with man. These tests are useful in the assessment of changes in pulmonary function in small animals. They are sensitive to small changes in lung structure, can be repetitively and routinely accomplished by a careful and competent researcher and the results appear to be highly reproducible.

This paper has been reviewed by the Health Effects Research Laboratory, United States Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Bates, D. V., Macklem, P. T., and Christie, R. V. Respiratory Function in Disease. W. B. Saunders, Philadelphia, 1971.
2. Comroe, J. H., Jr., Forster, R. E., II. DuBois, A. H., Briscoe, W. A., and Carlsen, E. The Lung. Year Book Medical Publishers, Chicago, 1965.
3. Mines, A. H. Respiratory Physiology. Raven Press, New York, 1981.
4. West, J. B. Respiratory Physiology—The Essentials. Williams and Wilkins, Baltimore, 1974.
5. West, J. B. Pulmonary Pathophysiology—The Essentials. Williams and Wilkins, Baltimore, 1977.
6. Leith, D. Comparative mammalian respiratory mechanics. Physiologist 19: 485–510 (1976).
7. King, T. K. C. Measurement of functional residual capacity in the rat. J. Appl. Physiol. 21: 233–236 (1966).
8. Takezawa, J., Miller, F. J., and O'Neil, J. J. Single breath diffusing capacity and lung volumes in small laboratory mammals. J. Appl. Physiol. 48: 1052–1059 (1980).
9. Palacek, F. Measurement of ventilatory mechanics in the rat. J. Appl. Physiol. 27: 149–156 (1969).
10. Koo, K. W., Leith, D. E., Sherter, C. B., and Snider, G. L. Respiratory mechanics in normal hamsters. J. Appl. Physiol. 40: 936–942 (1976).
11. Snider, G. L., Sherter, C. B., Koo, K. W., Karlinsky, J. B., Hayes, J. A., and Franzblau, C. Respiratory mechanics in hamsters following treatment with endotracheal elastase or collagenase. J. Appl. Physiol. 42: 206–215 (1977).
12. Snider, G. L., Celli, B. R., Goldstein, R. H., O'Brien, J. J., and Lucey, E. C. Chronic interstitial pulmonary fibrosis produced in hamsters by endotracheal bleomycin. Am. Rev. Respir. Dis. 117. 289–297 (1978).

13. Sinnett, E. E., Jackson, A. C., Leith, D. E., and Butler, J. P. Fast integrated flow plethysmograph for small mammals. J. Appl. Physiol. 50: 1104–1110 (1981).
14. Lai, Y.-L. Lung volumes and pleural pressure in the anesthetized hamster. J. Appl. Physiol. 46: 927–931 (1979).
15. Robertson, W. G., and Farhi, L. E. Rate of lung collapse after airway occlusion on 100% oxygen at various ambient pressures. J. Appl. Physiol. 20: 228–232 (1965).
16. Stengel, P. W., Frazer, D. G., and Weber, K. C. Lung degassing: an evaluation of two methods. J. Appl. Physiol. 48: 370–375 (1980).
17. Remmers, J. E., Gautier, H., and Bartlett, D. Factors controlling expiratory flow and duration. In: Loaded Breathing (L. D. Pengelly, A. S. Rebuck, E. J. M. Cambell, and D. M. Mills, Eds.), Langman, Ont., 1974, pp. 122–129.
18. Vinegar, A., Sinnett, E. E., and Leith, D. E. Dynamic mechanisms determine functional residual capacity in mice, *Mus musculus*. J. Appl. Physiol. 46: 867–871 (1979)
19. Raub, J. A., Mercer, R. R., Miller, F. J., Graham, J. A., and O'Neil, J. J. Dose response of elastase-induced emphysema in hamsters. Am. Rev. Respir. Dis. 125: 432–435 (1982).
20. Likens, S. A., and Mauderly, J. L. Effect of elastase or histamine on singie-breath N_2 washouts in the rat. J. Appl. Physiol. 52: 141–146 (1981).
21. Holub, D., and Frank, R. A system for rapid measurement of lung function in small animals. J. Appl. Physiol. 46: 394–398 (1979).
22. Fleming, G. M., Chester, E. H., Saniie, J., and Saidel, G. M. Ventilation inhomogeneity using multibreath nitrogen washout: comparison of moment ratios and other indexes. Am. Rev. Respir. Dis. 121: 789–794 (1980).
23. Saidel, G. M., Salmon, R. E., and Chester, H. B. Moment analysis of multibreath lung washout. J. Appl. Physiol. 38: 328–334 (1975).
24. Turek, Z., Kreuzer, F., and Ringnalda, B. E. M. Blood gases at several levels of oxygenation in rats with left-shifted blood oxygenation curve. Pflügers Arch. 376: 7–13 (1978).
25. Johanson, W. G., and Pierce, A. K. Lung structure and function with age in normal rats and rats with papain emphysema. J. Clin. Invest. 52: 2921–2927 (1973).
26. Crapo, J. D., Brody, A. R., Barry, B. E., and O'Neil, J. J. Morphologic, morphometric, and X-ray microanalytical studies on lung tissue of rats exposed to chrysotile asbestos in inhalation chambers. Proceedings International Agency for Research on Cancer, Lyon, 1979.
27. Gehr, P., O'Neil, J. J., Taylor, C. R., and Weibel, E. R. Discordant scaling between maximal O_2 consumption and pulmonary diffusing capacity in mammals. J. Physiol. 318: 648 (1981).
28. O'Brien, J. J., Lucey, E. C., and Snider, G. L. Arterial blood gases in normal hamsters at rest and during exercise. J. Appl. Physiol. 46: 806–810 (1979).
29. Lucey, E. C., O'Brien, J. J., Pereira, W., and Snider, G. L. Arterial blood gas values in emphysematous hamsters. Am. Rev. Respir. Dis. 121: 83–89 (1980).
30. Lai, Y.-L., Tsuya, Y., and Hildebrandt, J. Ventilatory responses to acute CO_2 in the rat. J. Appl. Physiol. 45: 611–618 (1978).
31. Rahn, H., Otis, A. B., Chadwick, L. E., and Fenn, W. O. The pressure-volume diagram of the thorax and lung. Am. J. Physiol. 146: 161–178. (1946).
32. Lucey, E. C., Celli, B. R., and Snider, G. L. Maximum expiratory flow and transpulmonary pressure in the hamster. J. Appl. Physiol. 45: 840–845 (1978).
33. Diamond, L., and O'Donnell, M. Pulmonary mechanics in normal rats. J. Appl. Physiol. 43: 942–948 (1977).
34. Lai, Y.-L., and Hildebrandt, J. Respiratory mechanics in the anesthetized rat. J. Appl. Physiol. 45: 255–260 (1978).
35. Freeman, G., Crane, S. C., Furiosi, N. J., Stephens, R. J., Evans, M., and Moore, W. D. Covert reduction in ventilatory surface in rats during prolonged exposure to subacute nitrogen dioxide. Am. Rev. Respir. Dis. 106: 563–579 (1972).
36. Hayatdavoudi, G., O'Neil, J. J., Barry, B. E., Freeman, B. A., and Crapo, J. D. Pulmonary injury in rats following continuous exposure to 60% oxygen for seven days. J. Appl. Physiol. 51: 1220–1231 (1981).
37. Young, S. L., Tierney, D. F., and Clements, J. A. Mechanism of compliance change in excised rat lungs at low transpulmonary pressure. J. Appl. Physiol. 29: 780–785 (1979).
38. Frazer, D. G., and Weber, K. C. Trapped air in ventilated excised rat lungs. J. Appl. Physiol. 40: 915–922 (1976).
39. Frazer, D. G., and Weber, K. C. Trapped gas at maximum lung volume in intact isolated rat lungs. Respir. Physiol. 37: 173–184 (1979).
40. Clements, J. A., and Tierney, D. F. Alveolar instability associated with altered surface tension. In: Handbook of Physiology, Section 3, Respiration. Vol. II (W. O. Fenn and H. Rahn, Eds.), American Physiological Society, Washington, 1964, pp. 1565–1583.
41. Mead, J. Analysis of the configuration of maximum expiratory flow volume curves. J. Appl. Physiol. 44: 156–165 (1978).
42. Damon, E. G., Mauderly, J. L., and Jones, R. K. Early effects of intratracheal instillation of elastase mortality, pulmonary morphology and respiratory function of Fischer-344 rats. Toxicol. Appl. Pharmacol. 64: 465–475 (1982).
43. Castile, G. C., Hyatt, R. E., and Rodarte, J. R. Determinants of maximal expiratory flow and density dependence in normal humans. J. Appl. Physiol. 49: 897–904 (1980).
44. Meadows, J. A., III, Rodarte, J. R., and Hyatt, R. E. Density dependence of maximal expiratory flow in chronic obstructive disease. Am. Rev. Respir. Dis. 121: 47–53 (1980).
45. Jackson, A. C., and Watson, J. W. Oscillatory mechanics of the respiratory system in normal rats. Respir. Physiol. 48: 309–322 (1982).
46. Vizek, M., and Palacek, F. Effect of anaesthesia on the pattern of breathing. Physiol. Bohemoslov. 26: 417–423 (1977).
47. Chapin, J. L. Ventilatory response of the unrestrained and unanesthetized hamster to CO_2. Am. J. Physiol. 179: 146–148 (1954).
48. Wong, K. L., and Alarie, Y. A method for repeated evaluation of pulmonary performance in unanesthetized, unrestrained guinea pigs and its application to detect effects of sulfuric acid mist inhalation. Toxicol. Appl. Pharmacol. 63: 72–90 (1982).
49. Lai, Y.-L., Lamm, W. J. E., and Hildebrandt, J. Ventilation during prolonged hypercapnia in the rat. J. Appl. Physiol. 51: 78–83 (1981).
50. Chvalova, M., Kuncova, M., Havrankova, J., and Palecek, F. Regulation of respiration in experimental silicosis. Physiol. Bohemoslov. 23: 539–547 (1974).
51. Gillespie, J., R., Bruce, E. Alexander, J., and Mead, J. Breathing responses of unanesthetized man and guinea pigs to increased transpulmonary pressure. J. Appl. Physiol. 47: 119–125 (1979).
52. Finucane, K. E., Egan, B. A., and Dawson, S. V. Linearity and frequency response of pneumotachographs. J. Appl. Physiol. 32: 121–126 (1972)
53. Fry, D. L. Physiologic recording by modern instruments with particular reference to pressure recording. Physiol. Rev. 40: 753–788 (1960).
54. Geddes, L. A., and Baker, L. E. Criteria for the faithful reproduction of an event. In: Principles of Applied Biomedical Instrumentation. John Wiley, New York, 1968, pp. 446–467.
55. Hök, B. Dynamic calibration of manometer systems. Med. Biol. Eng. 14: 193–198 (1976).
56. Jackson, A. C., and Vinegar, A. A technique for measuring frequency response of pressure, volume, and flow transducers. J. Appl. Physiol. 47: 462–467 (1979).
57. Leith, D. E., and Mead, J. Principles of Body Plethysmography. National Heart and Lung Institute, Bethesda, MD, 1974.
58. Proulx, P. A., Harf, A., Lorino, H., Atlan, G., and Laurent, D. Dynamic characteristics of air-filled differential pressure transducers. J. Appl. Physiol. 46: 608–614 (1979).
59. Drorbaugh, J. E., and Fenn, W. O. A barometric method for measuring ventilation in newborn infants. Pediatrics 16: 81–87 (1955)
60. Epstein M. A. F., and Epstein R. A. A theoretical analysis of the barometric method for measurement of tidal volume. Resp. Physiol. 32: 105–120 (1978).

61. Jacky, J. P. A plethysmograph for long-term measurements of ventilation in unrestrained animals. J. Appl. Physiol. 45: 644–647 (1978).
62. Jacky, P. J. Barometric measurement of tidal volume: effects of pattern and nasal temperature. J. Appl. Physiol. 49: 319–325 (1980).
63. Malan, A. Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. Respir. Physiol. 17: 32–44 (1973).
64. Pappenheimer, J. R. Sleep and respiration of rats during hypoxia. J. Physiol. 266: 191–207 (1977).
65. Frost, W. W. Analgesics, hypnotics, sedatives, and anesthetics used in laboratory animals. Health Sciences Resource Center, Univ. of Washington, Seattle, 1977, pp. 2–9.
66. Chenoweth, M. B. Inhalation anesthetics. Fed. Proc. 37: 2501–2503 (1978).
67. Agostoni, E., and D'Angelo, E. Comparative features of the transpulmonary pressure. Respir. Physiol. 11: 76–83 (1970).
68. Webb, H. H., and Tierney, D. F. Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. Am. Rev. Respir. Dis. 110: 556–565 (1974).
69. Kao, D. K., and Tierney, D. F. Air embolism with positive-pressure ventilation of rats. J. Appl. Physiol. 42: 368–371 (1977).
70. Seeherman, H. J., Taylor, C. R., Maloiy, G. M. O., and Armstrong, R. B. Measuring maximum aerobic capacity: pygmy mice to horses. Respir. Physiol. 44: 11–23 (1981).
71. O'Neil, J. J., and Leith, D. E. Lung diffusing capacity scaled in mammals from 25 g to 500 kg. Fed. Proc. 39: 972 (1980).
72. Spells, K. E. Comparative studies in lung mechanics based on a survey of literature data. Respir. Physiol. 8: 37–57 (1969).
73. Stahl, W. R. Scaling of respiratory variables in mammals. J. Appl. Physiol. 22: 453–460 (1967).
74. Tenny, S. M., and Remmers, J. E. Comparative quantitative morphology of the mammalian lung: diffusing area. Nature 197: 54–56 (1963).